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(54) IMPROVEMENTS IN OR RELATING TO THE PRODUCTION OF WORT

(71) We, SCHOLTEN - HONIG RESEARCH N.V. a Dutch Body Corporate, of P.O. Box 1, Foxhol, the Netherlands, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to the manufacture of wort or syrups for use in the fermentation industry. More particularly the invention relates to the use of corn steep water which has been subjected to ultrafiltration in the manufacture of wort or syrups.

Corn steep water, or corn steep liquor, is obtained as a by-product of the wet-milling process in the production of corn starch, when the grain is soaked in a warm dilute solution of sulphurous acid. During this process, which is referred to as steeping, a considerable amount of the acid soluble portion of the grain leaches into the steep water. Since steeping is normally accompanied by microbial fermentative conversion and degradation reactions, the steep water provides a rich source of nutrients, vitamins, minerals and unidentified growth factors, which can be usd to promote growth in micro-organisms, poultry, cattle and other animals. Besides the nitrogenous constituents, such as proteins, peptides and amino acids, other constituents such as sugars, lactic acid, phytic acid and minerals are present.

Although the steep water obtained by different starch wetmillers may show some differences in composition as a result of variations in the process, a typical average composition of dry substances in steep water is:

Total nitrogen	8%
Amino nitrogen	40—50% of total nitrogen
Lactic acid	20—25%
Phytic acid	6— 8%
Ash	18—20%

When such steep water is used as a nutrient in a fermentation industry, such as brewing, the results are not always satisfactory, because the balance between the constituents is not optimum. In U.S. patent no. 2,179,203 a process is described in which the heat coagulable proteins are removed from corn steep water by neutralisation and filtration. This treatment facilitates the use of corn steep water for fermentation purposes.

We have now found that when unneutralised or neutralised steep water is subjected to ultrafiltration, its composition can be changed in such way that its performance in various fermentation processes is still further improved.

Ultrafiltration is a process in which a solution is forced by a hydraulic pressure through a membrane or microporous filter thereby separating solution components on the basis of molecular size. Larger molecular species are retained by the membrane and collected in the form of a concentrated solution whereas most of the solvent and smaller solute species pass through the membrane and are collected as a permeate.

We have discovered that when corn steep water is subjected to ultrafiltration using filter material which are capable of retaining selected molecular species, such as proteins and peptides, thereby giving permeate and concentrate fractions wherein the relative amounts of proteins and amino acids have been altered, the suitability of these fractions for application in the manufacture of readily fermentable wort can be greatly improved.

In the manufacture of wort steepwater processed in this way can be used in admixture with high maltose syrups obtained by enzymatic and/or acid hydrolysis of protein containing amylaceous materials, such as cereal grains or cereal flours derived from unmalted barley, corn, wheat or rice. Other starch and protein containing materials such as tapioca flour or pure starches, such as root starch, tuber starch and cereal starch are also suitable.

According to the invention there is provided a process for the manufacture of readily fermentable wort or syrup, which comprises:

a) Subjecting steepwater originating from wet milling of corn which contains proteins, peptides and amino acids to ultrafiltration thereby obtaining a concentrate which is rich in proteins and a permeate which is rich in amino acids,

b) Solubilising and liquefying a mash of an amylaceous material with acid and/or enzyme followed by enzymatic saccharification to a starch conversion syrup containing more than 45% maltose by weight of total carbohydrates,

c) Mixing an appropriate amount of the corn steep water permeate fraction rich in amino acids and/or an appropriate amount of the corn steep water concentrate fraction rich in proteins either before or after the enzymatic saccharification with the liquefied amylaceous material to obtain a total nitrogen content in the mixture of 0.4—1% by weight of its dry substance, the nitrogeneous compounds being a mixture of proteins, peptides and amino acids, and adjusting, if necessary, the specific gravity of the mixture to that of wort by dilution or to that of wort syrup by concentration.

According to the present invention there is provided a process for the manufacture of readily fermentable wort or syrups as set forth in the preceding paragraph which further comprises, forming an aqueous mash of a protein-containing amylaceous material with a concentration in the order of 10—40% by weight, solubilising the starch and part of the protein by continuous cooking of the mash at a temperature of 100—150°C for a period of 1—10 minutes in the presence of an amount of a mineral acid which produces a pH level of 1.5 to 2.5, cooling and neutralising the mash and liquefying the starch with alphaamylase at temperatures of 40—105°C and a pH in the range of 4—7.5, separating the insoluble materials from the solution, adding an enzyme capable of breaking down starch to maltose and saccharifying the solution by maintaining the mixture at a controlled temperature for a period sufficient to form at more than 45% of maltose and adding before or after the saccharification step an appropriate amount of corn steep water permeate fraction rich in amino acids to obtain a total nitrogen content in the mixture of 0.4—1% by weight of its dry substance.

According to the present invention there is also provided a readily fermentable wort or syrup comprising a blend of an hydrolysate of amylaceous materials containing more than 45% of maltose based on the total carbohydrates, a permeate fraction rich in amino acids and/or a concentrate fraction rich in proteins obtained by ultrafiltration of corn steep water wherein the volume ratio of permeate fraction to concentrate fraction when both fractions are present is substantially different from the ratio in which they were obtained by ultrafiltration, which blend comprises 90—95% of carbohydrates, 0.4 to 1% of total nitrogen and 1 to 2% of ash based on the weight of the total solids, the total nitrogen being a mixture of proteins, peptides, and amino acids.

Further, according to the present invention there is provided a process of brewing beer comprising using a wort which is a blend of an hydrolysate of amylaceous materials containing more than 45% of maltose based on the total carbohydrates, a permeate fraction rich in amino acids and/or a concentrate fraction rich in proteins obtained by ultrafiltration of corn steep water, the amount of permeate fraction and/or concentrate fraction being chosen in such a way that the composition of the nitrogeneous compounds in this blend expressed as Lundin fractions is comparable to the composition of wort from malt.

In the process for the manufacture of wort or syrups according to the invention the optimum relative amounts of specific carbohydrates, proteins, peptides, amino acids, and other growth factors required in fermentation processes, such as brewing, is achieved by mixing appropriate amounts of the permeate containing mainly amino acids as nitrogenous compounds and/or appropriate amounts of concentrate containing mainly proteins with high maltose starch hydrolysate. Furthermore, this process offers a means of using relatively inexpensive materials like unmalted feed barley, wheat and corn, instead of the relatively expensive malt for fermentation processes, such as brewing of beer. In the literature processes have already been described for making brewery wort by treating a mash of cereal grain, which may be ground in different ways, with amylolytic and proteolytic enzymes. The drawbacks of these methods are:

a) The grain should be finely ground in order to obtain optimum enzymic hydrolysis, but this results in a very fine grist which causes difficulties in separation. Several solutions to this problem have been proposed, such as adding more proteolytic enzyme to allow the adoption of a coarser grist, or dehusking the grain before grinding and adding part of the husk fraction to the mash later. It is also possible to wet grind the grain and to add enzymes, such as hemicellulase, pentonase and pectinase, which degrade the cellular components hindering filtration. These precautions are unnecessary in the present process. Moreover, no proteolytic enzymes are required.

b) The extent of carbohydrate and protein

degradation in known enzymic worts is usually less than in wort made from malt. For this reason, additional coarse crushed malt has to be added to the grain grist. In the new process there is no need to add malt to the amylaceous material,

c) The ratio between the amino nitrogen and the total nitrogen in the known enzymic worts is not optimal, nor comparable with that of normal wort made from malt. In the processes of the present invention, the amino acids are supplied mainly by adding corn steep fractions, which can be varied by adjusting conditions in the ultrafiltration process, to give the ideal balance between the amino nitrogen and total nitrogen.

The high maltose syrups suitable for the process of the invention may be obtained by any known method of enzymatic and/or acid hydrolysis of mashes of amylaceous materials. In a preferred method for preparation of the high maltose syrups a grain or starch mash with a concentration in the order of 10 to 40% by weight is treated with a mineral acid in a continuous cooker.

Hydrochloric acid is the preferred mineral acid and the optimal amount used is that which produces a pH level of 1.5 to 2.5 in the mash. The continuous cooking procedure is performed at a temperature of 100—150°C for a period of 1—10 minutes. The aim of this treatment is to solubilise the starch and part of the protein to give a DE (Dextrose Equivalent) value of up to 25, but preferably of 15—20, and a soluble total nitrogen level, if protein containing raw materials are used, of up to 0.8% by weight of the total solids. This treatment also degrades the plant gums in such a way that their effect on filtration is substantially eliminated.

The starch mash is further liquified by treatment with a liquifying enzyme, such as a bacterial alpha-amylase, at temperatures of 40—105°C and a pH in the range of 4—7.5 and preferably at 75—95°C at a pH of about 6—6.5. The increase in DE during this step should be minimal and is usually not more than 3.

This liquefaction process may also be performed using other microbial alpha-amylases at other preferred temperature and pH levels.

In the separating step, which can be carried out before or after saccharification, sieving or filtering equipment or centrifuges may be used. Bent sieves, rotating sieves, filter presses and vacuum filters (provided with blades or strings) with or without filter aid, desludgers and decanters are examples of equipment suitable for this purpose. Generally, filtration is the process preferred.

In the saccharification step enzymes should be used which are capable of forming maltose. These may be enzymes with beta-amylase type activity of plant or microbial origin. It is also possible to use a microbial alpha-amylase in conjunction with an alpha, 1,6 glucosidase. In this case the alpha, 1,6 glucosidase may be derived from plants or from microbes such as bacteria of the genus *Pseudomonas* and *Aerobacter*. The conditions of pH and temperature should be selected in accordance with the optimum conditions for the specific enzyme. The maltose content of the saccharified solution should be more than 45% based on dry substance.

When starting from protein containing amylaceous materials, such as ground barley or corn, the total nitrogen content of the solution will be about 0.6% to 0.8% by weight of the dry substance. The composition of these nitrogenous compounds is treatment related, but it could be given approximately as:

Lundin A 52%; Lundin B 29%; Lundin C 19%.

Lundin fractions A, B and C, are brewery measurements, and refer to nitrogenous molecules of decreasing molecular weight. A typical ratio for malt worts is Lundin A 25%; Lundin B 15%; Lundin C 60%; the amino nitrogen is about 30% of the total nitrogen.

According to the invention the optimum Lundin ratio and amino nitrogen to total nitrogen ratio can be achieved by adding appropriate amounts of selected corn steep water permeate fractions and/or selected concentrate fractions obtained by membrane ultrafiltration.

The permeate fraction will contain most of the amino acids and the concentrate fraction most of the proteins, whereas the corn steep water peptides can, depending upon the choice of the membrane, be collected in the permeate, the concentrate or both. Hydrolysates made from protein containing amylaceous materials will need addition of corn steep water permeate to increase the Lundin C fraction. On the other hand to maltose containing hydrolysates from pure starch both permeate fraction and concentrate fraction will have to be added to obtain the proper amount of Lundin A, B and C fractions. Of course, the volume ratio of the added permeate and concentrate fractions must differ from the volume ratio in which they are obtained by ultrafiltration.

The mixture may be diluted to the required gravity and used directly as a wort for brewing purposes, or it may be concentrated to a syrup containing 70—80% dry substance. This syrup can be diluted to the required gravity in the breweries. The wort of the invention may be used as a complete or as a partial substitute for malt worts.

The invention also provides readily fer-

mentable wort or syrup when produced by our new process. These syrups therefore, consist of a blend of one or more fractions obtained by ultrafiltration of corn steep water using filter materials which are capable of substantially retaining proteins and peptides and of substantially passing amino acids, with hydrolysates of amylaceous materials containing maltose as the main sugar component, which blend consists of 90—95% of carbohydrates, 0.4 to 1% of total nitrogen and 1 to 2% of ash based on the weight of the total solids. More particular the wort or syrup may consist of

a) a blend of ultrafiltration permeate fraction of corn steep water and a hydrolysate of cereal grain or flour containing maltose as the main sugar component or

b) a blend of a major amount of the ultrafiltration concentrate fraction and a minor amount of the ultrafiltration permeate fraction of corn steep water and a hydrolysate of starch which contains maltose as the main sugar component.

The invention also provides a process for brewing beer in which the above worts or diluted syrups are used as raw materials.

The invention is illustrated in the following examples:

Example I

Neutralised corn steep water containing about 12% dissolved solids is ultrafiltrated through a cellulose acetate membrane which retains molecules with a molecular weight of more than 10,000 (e.g. the membrane sold under the Trade Name Amicon UM 10). At an average pressure of 15 atmospheres the flux is 20.0 litres per square metre of membrane per hour. An analysis of the concentrate and permeate collected is given in table A.

TABLE A:

Composition of corn steep water separa ted by ultrafiltration.

Composition of Total Solids in per cent:

	Feed	Concentrate	Permeate
Total nitrogen (T.N.)	8	8.3	7.4
Amino nitrogen / Total nitrogen ratio (per cent)	47	36	66
Lundin A Fraction (% T.N.)	18	29	0
Lundin B „ „	10	15	4
Lundin C „ „	72	56	96

Ground feed barley (containing appr. 12% protein corresponding with about 1.9% of total nitrogen) is slurried with water to a concentration of 25%. After adding hydrochloric acid to bring the pH to 1.8—2.0, the mixture is cooked in a continuous convertor at a temperature of 125°C for a period of 4 minutes. The mixture obtained after expansion, which has a DE of 12—14, is neutralised to pH 6.0—6.5 and treated with an alpha-amylase from Bacillus subtilis for 60 minutes at a temperature of 90°C. After cooling the mixture is filtered on a suction filter. The residue represents about 25% by weight of the barley and contains 5.3% by weight of total nitrogen. The filtrate is mixed with the ultrafiltration permeate fraction of corn steep water in the ratio of 5 parts of permeate (dry substance) per 100 parts of dry substance in the barley hydrolysate. This mixture is saccharified with a fungal alpha-amylase at a temperature of 50—55°C and at a pH of 5.3 for a period of 16 hours. After passage through a polishing filter, the saccharified product may be diluted and used as a wort for fermenting beer. In our case, however, the solution is first concentrated in vacuum to a syrup with 80% of dry solids having the composition given in table B.

TABLE B:

Carbohydrates	91.9%	based on Total Solids)
Containing glucose	9.9%	„
maltose	52.3%	„
maltotriose	15.2%	„
Total nitrogen	0.8%	„
Lundin A fraction	26 %	(based on Total Nitrogen)
B fraction	17 %	„
C fraction	57 %	„

After dilution with water this syrup is used for brewing beer. When beer prepared from this syrup is tested by a taste panel, the general opinion is very favourable.

Example II:

A corn starch syrup is prepared by a known method in which a starch slurry is liquified with a bacterial alpha-amylase and then saccharified with a beta-amylase. The average carbohydrate composition of a syrup prepared in this way as based on total solids:

Dextrose	6%
maltose	58%
maltotriose	12%
higher sugars	23%

Corn steep water is subjected to ultrafiltration to give concentrated permeate fractions with a composition similar to that in Table A.

To adjust the nitrogenous composition, these fractions are added in the ratio of 8 parts by weight of concentrate, 2 parts by weight of permeate and 90 parts by weight of the liquified and saccharified corn syrup (calculated as dry solids), to bring the total nitrogen level of the dry substance in the mixture to 0.8%.

This mixture is diluted to give a readily fermentable wort suitable for brewing beer. The Lundin fractions in this wort are comparable to those given in Example I. By varying the proportion of concentrate and permeate and the conditions in ultrafiltration, however, it is possible to adjust the protein composition to meet the special requirements of any particular fermentation process.

WHAT WE CLAIM IS:—

1. A process for the manufacture of readily fermentable wort or syrup, which comprises:

a) Subjecting steepwater originating from wet milling of corn which contains proteins, peptides and amino acids to ultrafiltration thereby obtaining a concentrate which is rich in proteins and a permeate which is rich in amino acids,

b) Solubilising and liquefying a mash of an amylaceous material with acid and/or enzyme followed by enzymatic saccharification to a starch conversion syrup containing more than 45% maltose by weight of total carbohydrates,

c) Mixing an appropriate amount of the corn steep water permeate fraction rich in amino acids and/or an appropriate amount of the corn steep water concentrate fraction rich in proteins either before or after the enzymatic saccharification with the liquefied amylaceous material to obtain a total nitrogen content in the dry mixture of 0.4—1% by weight of its dry substance, the nitrogenous compounds being a mixture of proteins, peptides and amino acids, and adjusting, if necessary, the specific gravity of the mixture to that of wort by dilution or to that of wort syrup by concentration.

2. A process as claimed in claim 1 which comprises forming an aqueous mash of a protein-containing amylaceous material with a concentration in the order of 10—40% by weight, solubilising the starch and part of the protein by continuous cooking of the mash at a temperature of 100—150°C for a period of 1—10 minutes in the presence of an amount of a mineral acid which produces a pH level of 1.5 to 2.5, cooling and neutralising the mash and liquefying the starch with alphaamylase at temperatures of 40—105°C and a pH in the range of 4—7.5, separating the insoluble materials from the solution, adding an enzyme capable of breaking down starch to maltose and saccharifying the solution by maintaining the mixture at a controlled temperature for a period sufficient to form more than 45% of maltose and adding before or after the saccharification step an appropriate amount of corn steep water permeate fraction rich in amino acids to obtain a total nitrogen content in the mixture of 0.4 to 1% by weight of its dry substance.

3. A process as claimed in claim 2, in which the protein containing amylaceous material to be hydrolysed is a cereal grain or a cereal flour.

4. A process as claimed in claim 3, in which the cereal is unmalted barley, corn, wheat or rice.

5. A process as claimed in claim 1, in which a hydrolysate of starch which contains maltose as the main sugar component is admixed with a major amount of the ultrafiltration concentrate fraction and a minor amount of the ultrafiltration permeate fraction of corn steep water to give a total nitrogen content of 0.4 to 1% by weight based on the dry substance of the final mixture.

6. A process as claimed in any of claims 1 to 5 in which the mixture is concentrated to a syrup containing 70—80% dry substance.

7. A process for the manufacture of readily fermentable wort or syrup as claimed in claim 1 and substantially as hereinbefore described with particular reference to either of the foregoing Examples.

8. A readily fermentable wort or syrup whenever produced by a process as claimed in any of claims 1 to 7.

9. A readily fermentable wort or syrup comprising a blend of an hydrolysate of amylaceous maerials containing more than 45% of maltose based on the total carbohydrates, a permeate fraction rich in amino acids and/

or a concentrate fraction rich in proteins obtained by ultrafiltration of corn steep water wherein the volume ratio of permeate fraction to concentrate fraction when both fractions are present is substantially different from the ratio in which they were obtained by ultrafiltration, which blend comprises 90—95% of carbohydrates, 0.4 to 1% of total nitrogen and 1 to 2% of ash based on the weight of the total solids, the total nitrogen being a mixture of proteins, peptides and amino acids.

10. A readily fermentable wort or syrup as claimed in claim 9 which comprises a blend of ultrafiltration permeate fraction of corn steep water and a hydrolysate of cereal grain or flour containing maltose as the main sugar component.

11. A readily fermentable wort or syrup as claimed in claim 10 which comprises a blend of a major amount of the ultrafiltration concentrate fraction and a minor amount of the ultrafiltration permeate fraction of corn steep water and a hydrolysate of starch containing maltose as the main sugar component.

12. A readily fermentable wort or syrup as claimed in claim 9 and substantially as hereinbefore described with particular reference to either of the foregoing Examples.

13. A process of brewing beer comprising using a wort which is a blend of an hydrolysate of amylaceous materials containing more than 45% of maltose based on the total carbohydrates, a permeate fraction rich in amino acids and/or a concentrate fraction rich in proteins obtained by ultrafiltration of corn steep water, the amount of permeate fraction and/or concentrate fraction being chosen in such a way that the composition of the nitrogeneous compounds in this blend expressed as Lundin fractions is comparable to the composition of wort from malt.

14. A process of brewing beer according to claim 13 in which the nitrogenous compounds are composed of about 25% Lundin A fraction, about 15% Lundin B fraction and about 60% Lundin C fraction.

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